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In vitro effectiveness of some antifungal, plant extracts, and essential oils on vaginal candidiasis-recovered *Candida* species from Surman, Libya

Nawal Abdulwahhab Alfaytouri[1], Mohamed Ahmed Al-Ryani[2]*

[1]Department of Botany, Faculty of Science, Sabratha University, Libya.
[2]Department of Botany, Faculty of Science, Al-Zentan University, Libya.

ABSTRACT

Purpose: Vulvovaginal candidiasis is a dangerous condition that affects around 75% of all women at some point in their life. This study aimed to diagnosis of yeasts that cause vulvovaginal candidiasis in pregnant women, and studying the effect of some antifungal drugs, plant extracts, and essential oils on growth of *Candida* isolates causing the disease.

Materials and Methods: Mycological analysis of 210 samples collected from patients, using SDA and chromogenic media for yeast identification, and the well diffusion method to evaluated how five plant extracts, and 5 essential oils, as well as 3 antifungal drugs affected the development of 28 *Candida* isolates.

Results: According to their color on the chromogenic media 100 Candida isolates classified into 5 species, namely *C. albicans, C. glabtrata, C. krusei, C. parapsilosis, and C. tropicalis*. The results showed that thyme extract, basil oil, and thyme oil were the most effective natural antifungal against the tested isolates. Clotrimazole and Miconazole were the two medications that had the best effect against the tested *Candida* isolates.

Conclusion: Antifungal incorrect use contributes to the development of anti-fungal resistance and therefore does not give effective results. In vitro susceptibility testing to various antifungal agents (drugs, extracts and essential oils) is not only the key to successful treatment of a fungal disease, but also the gateway to the discovery of new useful antifungals. *(Open J Biomed Res 2023;2:16-25.)*

Keywords

Vulvovaginal candidiasis infections, antifungal agent, plant extracts, Essential oils, *Candida albicans*.

AUDIOABSTRACTT

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Corresponding Author:

Dr. Mohamed Al-Ryani

Department of Botany, Faculty of Science, Al-Zentan University, Libya.

Email: profdrnani@uoz.edu.ly

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INTRODUCTION

Yeasts are part of the normal microbiome of the vagina.[1,2] However, some species display an opportunistic behavior shifting from a non-pathogenic commensal microorganism to a tissue colonizing fungi leading to a variety of clinical manifestations in patients with predisposing factors.[3] A vaginal yeast infection usually commences when a change in the delicate balance in a woman's system occurs, as a result of pregnancy, diabetes or drug use like antibiotic or corticosteroid treatment.[4] Candidiasis has been defined since Hippocrates' (approximately 460–370 BC) time as "a sickness of the ill," emphasizing the opportunistic nature of the infection and the fundamental function of a weakened host defense in its development.[5] In the developed world, invasive candidiasis is the most frequent fungal illness among hospitalized patients. Candidaemia and deep-seated tissue candidiasis are both types of invasive candidiasis. Candidaemia is often regarded as the more prevalent form of the disease, accounting for the vast majority of patients included in research studies. Deep-seated candidiasis is caused by either hematogenous spread or direct injection of *Candida* species into a sterile location, such as the peritoneal cavity. Even when antifungal medication is used, mortality among individuals with invasive candidiasis can reach 40%. Furthermore, the worldwide shift toward non-albicans *Candida* species is concerning, as is the developing resistance to antifungal medications. New findings have significantly altered diagnostic and treatment procedures in recent years.[6] There are several reports recording differences in the types of *Candida* causing VVC and and their antifungal drug susceptibility

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patterns from different geographic locations.[7] *C. albicans* has been reported to be responsible even for 90% of VVC infections.[8] In the past decades, the incidence of infections caused by NCA species has increased significantly, especially recurrent infections.[9]

An antifungal agent is a drug that selectively eliminates fungal pathogens from a host with minimal toxicity to the host. The number of antifungal medications is restricted in comparison to antibacterial treatments since the fungus is a eukaryotic organism that parasitizes a eukaryotic host, and the small range of physiologic differences between them makes it difficult to design safe and broad-spectrum antifungal agents. There are just a few kinds of antifungal drugs that can be used to treat fungal infections due to toxicity and the development of treatment resistance.[10] Many plant species have been employed for medicinal purposes in the hunt for illness treatment and cure, a factor that has experimentally spread over the world and passed down from generation to generation.[11] In this regard, humans have demonstrated an interest in their well-being and quality of life, driving the general public and the scientific community to search and identify novel chemicals from plant species that are helpful to the human body.[12] Due to more aggressive and endogenous bacteria that are resistant to the use of synthetic antimicrobials, there is a rising desire to limit the use of chemicals as antimicrobial agents in the field of nutrition and to treat different illnesses. Plant-derived compounds, such as hydro-alcoholic extracts or essential oils, might undoubtedly play an important role in this regard. Because of their diverse chemical structures, such compounds have considerable adaptability; the same plant can offer a pool of chemicals with a very broad spectrum of activity.[13] Furthermore, hypersensitivity and toxicity to medications as a result of incorrect and excessive application are some of the key difficulties in traditional medicine as a result of the currently excessive usage of synthetic antimicrobials. As a result, public awareness has sparked interest in the use of natural compounds that have been used for centuries to cure illnesses spread by organisms. Essential oils have been used since the dawn of civilization, first in the East and Middle East, then in North Africa and Europe.[14] One of the most promising natural compounds for inhibiting fungi might be essential oils (Eos). Actually, several EO varieties made from various plats or herbs displayed potent antifungal effects similar to other phytochemicals, Eos may inhibit the formation of biofilms and microbial growth through particular mechanisms.[15,16,17] Therefore, This study aimed to diagnosis of yeasts that cause vulvovaginal candidiasis in pregnant women, and studying the effect of some antifungal drugs, plant extracts, and essential oils on growth of *Candida* isolates causing the disease.

MATERIALS AND METHODS

Collection of samples

This study included 210 specimens from vaginal yeast infections collected from patients admitted to the Maternity Care Center in the combined clinic, Surman, Libya, during the period from June to December 2022. Samples were taken by sterile swabs under complete aseptic precautions in sterile containers from the patient's vagina. These samples were handled with caution to avoid sample contamination or dissemination of infectious agents and carried immediately to the Microbiology Laboratory in the National Cancer Institute, Subrata, Libya for direct microscopy examination (DME), culturing, and characterization.

Direct microscopic examination (DME)

From each vaginal swab, two slides were prepared, one stained with Gram stain (GS) and the other with lactophenol cotton blue (LPCB) stain. The light microscope was used to examine the slides at 40x and 100x magnifications. Positive yeast infection findings were obtained when samples showed budding yeast cells with or without pseudohyphae.

Culturing of specimens

To identify mixed infections, swabs were streaked over the surface of Sabouraud's dextrose agar (SDA), supplemented with chloramphenicol 250 mg/l and 0.5 g/l cycloheximide to reduce bacteria and saprophytic fungi. Cultures were incubated at 37°C for 24-96 hours. A microscopic inspection of colony development was performed to establish the existence of fungal units. The developing fungi were kept in SDA slants for further study.

Culturing on HiCrome *Candida* agar

HiCrome *Candida* differential agar (HiMedia Company, India) is a selective and differential medium that allows for the rapid isolation of yeasts from mixed cultures and the differentiation of *Candida* species based on coloration and colony morphology. The results are received in 48 hours and are useful for the quick and preliminary identification of common yeasts in Mycology and Clinical Microbiology Laboratories. *C. albicans* has light green-colored smooth colonies, while *C. tropicalis* has blue to metallic blue-colored elevated colonies, according to the manufacturer. Colonies of *C. glabrata* have a creamy to white smooth appearance, whilst those of Issatchenkia orientalis have a purple fuzzy appearance. All the *Candida* isolates were deposited in the culture collection of the Assiut University Mycological Centre (AUMC) Assiut, Egypt.

Antifungal susceptibility test

The disk diffusion method adopted by Clinical and Laboratory Standards Institute (CLSI) was used to evaluate the degree of fungal sensitivity to miconazole, clotrimazole, fluconazole, basil oil, Fenugreek oil, marjoram oil, Rosemary oil, thyme oil, marjoram extract, basil extract, Rosemary extract, thyme extract, and mint extract. All antifungal discs were obtained from HiMedia Company, India. Sabouraud's dextrose agar (SDA) was used for monitoring the antifungal activity against Candida species.[18-20]

Preparation of inoculums

The spore suspensions were made from cultures cultivated on SDA for 24 hours. Colonies were suspended in a sterile saline solution of 2 ml (0.85 %). The suspension was vortexed to homogenize it, and the turbidity was adjusted to produce 1.5 × 105 cells/ml (= 0.5 McFarland standard solution).

Susceptibility test procedure

The antifungal activity of different antifungal standards, plant methanolic extracts and oils against *Candida* species was evaluated by agar well diffusion,[21] using Sabouraud's dextrose agar (SDA). The *Candida* species tested were activated by inoculating a loopful of each strain in the Sabouraud's broth and incubated at 28 ºC for 24 h. Then 0.2 ml of inoculum (inoculum size was 108cells/ml as per McFarland standard) was inoculated into the molten SDA and after proper homogenization it was poured into 10 cm Petri dishes. For the agar well diffusion, wells were made in the seeded plates with the help of a cup-borer (5.0 mm diameter). The test compounds were introduced separately into the wells and all the plates were incubated at 28 ºC for 48 h. The experiment was performed under strict aseptic conditions. Microbial growth was determined by measuring the diameter of the zone of inhibition and the mean values were presented and compared with the standard antifungal agents (Table 1).

Preparation of plant extract:

Plant samples and plant oils

Fresh plants/plant parts were collected in May 2021 to January 2022, from Alryana, Aljabal Algarbi - Libya. Fresh plant material was washed under running tap water, air dried, and then homogenized to fine powder and stored in airtight bottles. Oils of Basil, Fenugreek, Marjoram, Rosemary, and Thyme were obtained from Faculty of Agriculture, Assiut University, Egypt.

Methanol extraction

A 100 g dried plant material of five plants namely Basil (Ocimum basilicum), Marjoram (Origanum majorana), Mint (Mentha arvensis), Rosemary (Rosmarinus officinalis), and Thyme (Thymus vulgaris) were separately extracted three times with 500 ml of methanol on a water bath at 50 ºC for 60 minutes each time. Following that, it was filtered and centrifuged at 5,000 rpm for 20 minutes. The supernatant was collected, and the solvent was evaporated, resulting in a final volume one-fifth of the initial volume.[22,23] The extracts were airdried till they reached powder form.

RESULTS

Isolation of *Candida* species

During the present study, it was possible to examine 210 patients complaining of yeast vaginal infections. Mycological analysis of all collected samples revealed the isolation of 100 strains belonging to genus *Candida*.

Identification of *Candida* species using Chrome agar

A total of 100 isolates have been recovered in this study, of which 72 isolates were obtained as pure cultures on Chrome agar medium. According to their color on Chrome agar, these 72 colonies were categorized to 5 main species namely *Candida albicans* (Robin) Berkhout, *C. glabrata* (Anderson) Meyer & Yarrow, *C. krusei* (Castell) Berkhout, *C. parapsilosis* (Ashford) Langeron & Talice, and *C. tropicalis* (Ashford) Langeron & Talice.

Effect of some standard antifungal compound on *Candida* growth

All of the *C. krusei* isolates under examination, with the exception of one, exhibited Fluconazole resistance. While Clotrimazole and Miconazole were the two medications that the studied isolates reacted to the best. It affected *C. albicans* isolates by 15 to 28 mm, *C. krusei* by 17 to 28 mm, *C. glabrata* by 17 to 26 mm, *C. parapsilosis* by 20 to 23 mm, and *C. tropicalis* by 25 mm. 11 of the 28 isolates showed Miconazole resistance. They were 5 *C. krusei*, 2 *C. glabrata*, 2 *C. parapsilosis*, 2 *C. albicans*, and 2 *C. krusei*. Miconazole demonstrated a range of effects on *C. albicans*, including11-19 mm, 11-15 mm, 15-22 mm, and 25 mm versus *C. tropicalis* (Table 2).

Table 1. Interpretative breakpoints of antifungal agents.

Antifungal	Dose/disc	Zone diameter in mm		
		S	I	R
Nystatin (NS)	100 U	≥ 15	10 – 14	≤ 9
Amphotericin B (AP)	100 U	≥ 15	10 – 14	≤ 9
Fluconazole (FLC)	25 µg	≥ 19	15 – 18	≤ 14
Itraconazole (IT)	10 µg	≥ 23	14 – 22	≤ 13
Clotrimazole (CC)	10 µg	≥ 20	12 – 19	≤ 11
Ketoconazole (KT)	10 µg	≥ 28	27 – 21	≤ 20
Miconazole (MIC)	10 µg	≥ 20	12 – 19	≤ 11
Voriconazole (VRC)	1 µg	≥ 17	14 – 16	≤ 13

S: Sensitive, I: Intermediate, R: Resistant.

Table 2. Effect of some standard antifungal compounds(expressed in mm inhibition zone) on the growth of *Candida* isolates recovered from vaginal infections($p \leq 0.05$).

***Candida* isolates**	**Miconazole**	**Clotrimazole**	**Fluconazole**
*C. albicans*1	19 ^{c}	24 ^{de}	0
*C. albicans*2	14 ^{hi}	22 ^{ef}	0
*C. albicans*3	11 ^{k}	18 ^{g}	0
*C. albicans*4	0 ^{l}	15 ^{h}	0
*C. albicans*5	17 ^{def}	23.33 ^{def}	0
*C. albicans*6	18.33 ^{cd}	18 ^{g}	0
*C. albicans*7	0 ^{l}	23 ^{def}	0
*C. albicans*8	16 ^{fg}	18 ^{g}	0
*C. albicans*9	15 ^{gh}	28 ^{a}	0
*C. albicans*10	13 ^{ij}	23 ^{def}	0
*C. krusei*1	0 ^{l}	17 ^{gh}	13 ^{ij}
*C. krusei*2	0 ^{l}	17 ^{gh}	0
*C. krusei*3	16.33 ^{efg}	25 ^{cd}	0
*C. krusei*4	11 ^{k}	23 ^{def}	0
*C. krusei*5	15 ^{lgh}	23 ^{def}	0
*C. krusei*6	11 ^{k}	26 ^{bc}	0
*C. krusei*7	0 ^{l}	25 ^{de}	0
*C. krusei*8	12 ^{jk}	27 ^{ab}	0
*C. krusei*9	0 ^{l}	28 ^{a}	0
*C. krusei*10	0 ^{l}	22 ^{ef}	0
*C. glabrata*1	0 ^{l}	17 ^{gh}	0
*C. glabrata*2	22 ^{b}	22 ^{ef}	0
*C. glabrata*3	18 ^{jk}	17 ^{h}	0
*C. glabrata*4	0 ^{l}	18 ^{g}	0
*C. glabrata*5	15 ^{gh}	26 ^{bc}	0
*C. parapsilosis*1	0 ^{l}	20 ^{f}	0
*C. parapsilosis*2	0 ^{l}	23 ^{def}	0
*C. tropicalis*1	25 ^{a}	25 ^{bc}	0

At the 0.05 level of probability, means in a column with the same letters are not statistically different.
a, b, c, d, …etc Indicates the significant differences between the values in each column.

Effect of some plant extract on *Candida* growth

Using the well diffusion method, it was evaluated how five plant extracts affected the development of 28 *Candida* isolates from the species *C. albicans* (10 isolates), *C. glabrata* (5), *C. krusei* (10 isolates), *C. parapsilosis* (2), and *C tropicalis* (1). The most effective treatment for all the tested *Candida* isolates, according to the current findings, was thyme extract. Thyme extract had a range of effects on *Candida*: 10-12 mm, inhibitory zone for Candida albicans, 7-8 mm for *Candida* krusei, 12-13 mm for *Candida* glabrata, 9-10 mm for *Candida* parapsilosis, and 10 mm for *Candida* tropicalis. Only marjoram and basil each on a single *C. albicans* isolate had any impact on the other extracts (Table 3).

Effect of some plant oils on *Candida* growth

Using the well diffusion method, it was evaluated how five plant oils affected the development of 28 *Candida* isolates from the species *C. albicans* (10 isolates), *C. glabrata* (5), *C. krusei* (10 isolates), *C. parapsilosis* (2), and *C. tropicalis* (1). The most effective oil for all the tested *Candida* isolates, according to the current findings, was basil oil followed by thyme oil. Basil oil had a range of effects on *Candida*: 13-23 mm inhibitory zone for *Candida albicans*, 13-24 mm for *Candida krusei*, 8-19 mm for *Candida glabrata*, 9 mm for *Candida parapsilosis*, and 13 mm for *Candida tropicalis* (Table 4).

Table 3. Effect of five plant extracts (expressed in mm inhibition zone) on the growth of *Candida* isolates recovered from vaginal infections($p \leq 0.05$).

Candida isolates	Marjoram extract	Basil extract	Rosemary extract	Thyme extract	Mint extract
*C. albicans*1	0 ^{n}	0	0 ^{b}	12.33 ^{b}	0 ^{i}
*C. albicans*2	0 ^{n}	0	0 ^{b}	12.33 ^{b}	0 ^{i}
*C. albicans*3	10.33 ^{k}	9 ^{g}	0 ^{b}	11.00 ^{de}	0 ^{i}
*C. albicans*4	0 ^{n}	0	0 ^{b}	10.33 ^{e}	0 ^{i}
*C. albicans*5	0 ^{n}	0	0 ^{b}	12.33 ^{b}	0 ^{i}
*C. albicans*6	0 ^{n}	0	0 ^{b}	11.00 ^{de}	0 ^{i}
*C. albicans*7	0 ^{n}	0	0 ^{b}	11.33 ^{cd}	0 ^{i}
*C. albicans*8	0 ^{n}	0	0 ^{b}	12.00 ^{bc}	0 ^{i}
*C. albicans*9	0 ^{n}	0	0 ^{b}	10.33 ^{e}	0 ^{i}
*C. albicans*10	0 ^{n}	0	0 ^{b}	10.66 ^{de}	0 ^{i}
*C. krusei*1	0 ^{n}	0	0 ^{b}	8.00 ^{fg}	0 ^{i}
*C. krusei*2	0 ^{n}	0	0 ^{b}	7.33 ^{g}	0 ^{i}
*C. krusei*3	0 ^{n}	0	0 ^{b}	7.33 ^{g}	0 ^{i}
*C. krusei*4	0 ^{n}	0	0 ^{b}	7.33 ^{g}	0 ^{i}
*C. krusei*5	0 ^{n}	0	0 ^{b}	7.33 ^{g}	0 ^{i}
*C. krusei*6	0 ^{n}	0	0 ^{b}	7.33 ^{g}	0 ^{i}
*C. krusei*7	0 ^{n}	0	0 ^{b}	7.33 ^{g}	0 ^{i}
*C. krusei*8	0 ^{n}	0	0 ^{b}	7.33 ^{g}	0 ^{i}
*C. krusei*9	0 ^{n}	0	0 ^{b}	7.33 ^{g}	0 ^{i}
*C. krusei*10	0 ^{n}	0	0 ^{b}	7.33 ^{g}	0 ^{i}
*C. glabrata*1	0 ^{n}	0	0 ^{b}	12.00 ^{bc}	0 ^{i}
*C. glabrata*2	0 ^{n}	0	0 ^{b}	12.33 ^{b}	0 ^{i}
*C. glabrata*3	0 ^{n}	0	0 ^{b}	12.00 ^{bc}	0 ^{i}
*C. glabrata*4	0 ^{n}	0	0 ^{b}	12.00 ^{bc}	0 ^{i}
*C. glabrata*5	0 ^{n}	0	0 ^{b}	13.33 ^{a}	0 ^{i}
*C. parapsilosis*1	0 ^{n}	0	0 ^{b}	8.66 ^{f}	0
*C. parapsilosis*2	0 ^{n}	0	0 ^{b}	10.33 ^{e}	0 ^{i}
*C. tropicalis*1	0 ^{n}	0	0 ^{b}	10.33 ^{e}	0 ^{i}

At the 0.05 level of probability,means in a column with the same letters are not statistically different.
a, b, c, d, …etc Indicates the significant differences between the values in each column.

Discussion

The present study revealed that patients who complained of vulvovaginal candidiasis (VVC) frequently had yeast infections. In vitro testing was done to determine if yeasts were susceptible to azole antifungal drugs. Also, the tests employed some plant oils and water extracts. In the current study, 210 women, from Surman, Libya were clinically diagnosed with VVC, of which 71 revealed positive direct microscopic examination (DME) accounting for 33.8 % of the total. Budding cells and/or pseudohyphae in the examined samples were considered as indications of the positive results. Culturing on specific media demonstrated positive results in 100 cases constituting 47.62 % of the total cases. In this investigation, the most

Table 4. Effect of five plant oils (expressed in mm inhibition zone) on the growth of Candida isolates recovered from vaginal infections($p \leq 0.05$).

Candida isolates	Basil oil	Peppermint oil	Marjoram oil	Rosemary oil	Thyme oil
*C. albicans*1	21.66 ^{c}	0 ^{h}	0 ^{b}	0 ^{h}	12.66 ^{b}
*C. albicans*2	17.33 ^{f}	0 ^{h}	2.33 ^{a}	0 ^{h}	12 .00 ^{bc}
*C. albicans*3	13.66 ^{hi}	0 ^{h}	0 ^{b}	0 ^{h}	11.33 ^{cd}
*C. albicans*4	13.00 ^{ij}	0 ^{h}	0 ^{b}	0 ^{h}	8.66 ^{g}
*C. albicans*5	21.66 ^{c}	0 ^{h}	2.33 ^{a}	0 ^{h}	12.66 ^{b}
*C. albicans*6	18.33 ^{de}	0 ^{h}	0 ^{b}	0 ^{h}	11.33 ^{cd}
*C. albicans*7	17.33 ^{f}	0 ^{h}	0 ^{b}	0 ^{h}	10.33 ^{ef}
*C. albicans*8	14.66 ^{g}	0 ^{h}	0 ^{b}	0 ^{h}	11.33 ^{cd}
*C. albicans*9	14.33 ^{gh}	0 ^{h}	0 ^{b}	0 ^{h}	12.66 ^{b}
*C. albicans*10	22.66 ^{b}	0 ^{h}	0 ^{b}	0 ^{h}	12.66 ^{b}
*C. krusei*1	13.33 ^{ij}	0 ^{h}	0 ^{b}	0 ^{h}	10.66 ^{de}
*C. krusei*2	15.00 ^{g}	0 ^{h}	0 ^{b}	0 ^{h}	12.33 ^{b}
*C. krusei*3	13.33 ^{ij}	0 ^{h}	0 ^{b}	0 ^{h}	12.66 ^{b}
*C. krusei*4	21.66 ^{c}	0 ^{h}	0 ^{b}	0 ^{h}	12.66 ^{b}
*C. krusei*5	13.66 ^{hi}	0 ^{h}	0 ^{b}	0 ^{h}	12.33 ^{b}
*C. krusei*6	17.66 ^{ef}	0 ^{h}	0 ^{b}	0 ^{h}	12.66 ^{b}
*C. krusei*7	13.66 ^{hi}	0 ^{h}	0 ^{b}	0 ^{h}	9.66 ^{f}
*C. krusei*8	24.33 ^{a}	0 ^{h}	0 ^{b}	0 ^{h}	13.66 ^{a}
*C. krusei*9	17.66 ^{ef}	0 ^{h}	0 ^{b}	0 ^{h}	12.33 ^{b}
*C. krusei*10	12.66 ^{j}	0 ^{h}	0 ^{b}	0 ^{h}	10.66 ^{de}
C. glabrata 1	10.33 ^{k}	0 ^{h}	0 ^{b}	0 ^{h}	7.33 ^{h}
C. glabrata 2	7.666 ^{m}	0 ^{h}	0 ^{b}	0 ^{h}	7.33 ^{h}
C. glabrata 3	7.666 ^{m}	0 ^{h}	0 ^{b}	0 ^{h}	7.33 ^{h}
C. glabrata 4	7.666 ^{m}	0 ^{h}	0 ^{b}	0 ^{h}	7.33 ^{h}
C. glabrata 5	18.66 ^{d}	0 ^{h}	0 ^{b}	0 ^{h}	12.66 ^{b}
*C. parapsilosis*1	8.66 ^{l}	0 ^{h}	0 ^{b}	0 ^{h}	8.66 ^{g}
*C. parapsilosis*2	8.66 ^{l}	0 ^{h}	0 ^{b}	0 ^{h}	8.66 ^{g}
C. tropicalis 1	14.00 ^{j}	0 ^{h}	0 ^{b}	0 ^{h}	11.33 ^{cd}

At the 0.05 level of probability,means in a column with the same letters are not statistically different.
a, b, c, d, …etc Indicates the significant differences between the values in each column.

common species isolated from women with VVC, was *C. albicans*. *Candida albicans* predominated in most cases. The development of *C. albicans* may be explained by the presence of vaginal cell receptors, which make it easier for this particular species of *Candida* to attach to the vaginal mucosa, allowing them to germinate and change from pathogenic blastospores to filamentous forms 24, 25. Additionally, non-*Candida albicans* (NCA) contributed to roughly 20.0 % of all cases, according to the data from the current study. As a result, several investigations in recent decade have noted a gradual shift in the prevalence of yeast infections away from *C. albicans* and towards NCA species26. Accordingly, comparable investigations carried out in Egypt found that the frequency of NCA ranged from 21.7 % to 54.8 %,[27-29] and reached 53.0% in India.[30]

Candida glabrata, C. krusei, C. parapsilosis, and *C. tropicalis* were isolated from VVC less often after *C. albicans* in the current investigation. In this regard, numerous investigations have demonstrated that these species coexisted with C. albicans in vaginal yeast infections, which is consistent with the present findings.[31-33] The incidence of VVC varies amongst nations in general, with a range of incidence between 20.0 % and 75.0 %.[31,34-37] However, according to many studies conducted in Egypt, the prevalence of VVC infections ranged from 49%-64%.[27-29] According to reports, one of the reasons of recurring VVC and antifungal drug resistance is NCA infection.[28] In Egypt, samples taken from long-term steroid users who complained of vulvovaginal candidiasis included 48.0 % of NCA.[38] Nearly the same percentage of NCA (47.1%) was observed among HIV-positive women in Brazil.[39] This high incidence of NCA may be explained by the fact that chronic steroid users typically have more frequent VVC episodes that need for more frequent empirical antifungal therapy, which contributes to the switch from *C. albicans* to NCA forms of yeast infections.[40,41] Additionally, frequent use of antifungal medication alters colonization patterns, enabling a rise in the prevalence of NCA species in appreciable numbers.[42]

In this study, the sensitivity to fluconazole, clotrimazole, and miconazole of a total of 28 yeast strains was determined, 39.0 % and 96.43 % of the tested yeast strains were resistant to miconazole and fluconazole, respectively. On the other hand, clotrimazole was the best effective antifungal against all tested strains. All but one of the *C. krusei* isolates under investigation displayed fluconazole resistance. The examined isolates responded most favorably to the drugs clotrimazole and miconazole. In this sense, [43] reported that, in comparison to azoles, amphotericin B and nystatin were more effective against Candida strains than azole compounds. Additionally, it was shown that 97.3 % of the *Candida strains from diverse* clinical specimens were amphotericin B- and nystatin-susceptible.[44]

Numerous studies revealed that the most frequent risk factors for azoles resistance among Candida strains of VVC patients were extended treatment and increasing antifungal usage.[45,46] However, resistant strains of several antifungal drugs are becoming more prevalent, especially for azoles and echinocandins.[47] Additionally, the incorrect use of antifungal medications and the introduction of over-the-counter antimycotics in many nations throughout the world contribute to the development of antifungal resistance.[48] The genetic changes (Up-regulation of ERG 11 gene coding for lanosterol 14-demethylase and CDR1 gene coding for Efflux pump) caused by the entry of steroid drugs into the fungal cell were thought to be responsible for the increase in yeast cell resistance. These genetic modifications help the yeast resistance to antifungal agents especially azole compounds.[49] This rise in azole resistance is consistent with earlier research by,[50] who showed that patients using itraconazole prophylaxis and other previously given azole medications including ketoconazole and miconazole had developed cross-resistance to fluconazole.[41]

The current results showed a highly significant resistance of *Candida* strains tested to fluconazole compared with miconazole and clotrimazole. One of the following explanations might be used to explain this resistance; the first one is related to the increased prevalence of *C. albicans* as a normal component of the human body's mycobiota causes it to be more frequently subjected to prophylaxis treatments, which increases its cross-resistance to azole medications. Additionally, because infections are more common, *C. albicans* is also more frequently subjected to azole treatment. In this context, it has reported instances of species-specific azole resistance in Brazil.[51] It has been demonstrated that resistance to one antifungal medication causes cross-resistance to medications in the same class.[52] However, this was not clear in the present study.

In the current study, the effect of five essential oils on growth of 28 *Candida* strains was assessed. The most effective oil for all the tested *Candida* strains, according to the current findings, was basil oil followed by thyme oil. Linalool and estragole (methyl chavicol), as well as o-cymene, citral, alpha-pinene, camphene, beta-pinene, geraniol, and geranial, are the two primary components of the 0.2–1% essential oil found in basil leaves.[53] According to genetic characteristics, regional origins, nutritional quality, the plant parts that were harvested (stem, leaf, and flower), as well as the extraction processes, the main basil oil constituents vary greatly.[54] However, due to changes in the content of the plant and oil, a number of chemotypes have been identified using the primary ingredients linalool and estragole, either individually

or in combination, as well as with linalool and eugenol or estragole and methyl eugenol.[55]

There are two main types of basil oil, which are widespread in trade: the Reunion Type, mostly composed of estragole (80%), and the European Type coming from France, Italy, Egypt, and South Africa, which is mostly composed of linalool (35-50%) and estragole (15-25%).[56] The use of basil as an analgesic, an anti-inflammatory, and for the alleviation of symptoms including cough, indigestion, cephalalgia, diarrhea, and constipation has a long history in traditional medicine.[57] One of the top ten most popular oils in the world, thyme oil is used as a natural food preservative, has potent antioxidant, antibacterial, and antifungal properties, and adds flavor to a variety of foods and beverages as well as personal care products like perfumes, cosmetics, soaps, and oral solutions.[58] Due to more aggressive and endogenous bacteria that are resistant to the use of synthetic antimicrobials, there is a rising desire to limit the use of chemicals as antimicrobial agents in the field of nutrition and to treat different illnesses. Plant-based chemicals may undoubtedly play a significant role in this approach. Examples include hydro-alcoholic extracts and essential oils.[13] Such chemicals are incredibly versatile; due to their various chemical structures, a single plant may produce a variety of molecules with a wide range of therapeutic effects. Additionally, some of the primary issues with traditional treatment as a result of the current overuse of synthetic antimicrobials include hypersensitivity and drug toxicity due to inappropriate and excessive medication administration. Because of this, interest has been sparked in using natural remedies that have been utilized for centuries to cure some ailments brought on by organisms. Even in the oldest civilizations, essential oils were often used, first in the East and Middle East, then in North Africa and Europe.[14] Chemical composition of plant essential oils differ among species; it is affected by factors including the geographical location, environment, the stage of maturity and method of extraction. This chemical difference is directly correlated to differences in biological activities.[59] We recommend studying the effect of plant extracts that gave results on the fungi isolated from the vagina and using them as a treatment for patients and comparing the extent of their effectiveness inside the human body with the results we obtained in the laboratory.

Conclusion

This study examined the development of the *Candida* isolates responsible for vaginal candidiasis in expectant mothers. It also examined the effects of various antifungal medications, plant extracts, and essential oils. After 210 patient samples were subjected to mycological analysis, 100 *Candida* isolates were found. These isolates were divided into five species, namely *C. albicans, C. glabrata, C. krusei, C. parapsilosis*, and *C. tropicalis*. The impact of three antifungal medications, five plant extracts, and five essential oils on the growth of Candidaisolates. It was demonstrated that the best natural antifungals against the tested isolates were thyme extract, basil oil, and thyme oil. Miconazole and clotrimazole were the two drugs that most effectively combated the isolates of *Candida* that were the subject of the study.

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